both polymorphisms (–611A and –56C) was significantly higher in DNTM patients than in controls (OR=16.8, CI 95%=2.1–132), or in PNTM patients (OR=9.3, CI 95%=1.1–78.1). The probability of finding polymorphism –611A in PNTM was higher than in controls (OR=8.4, CI 95%=2.4-29.4), but not different than in DNTM (OR=1.3, CI 95%=0.1–14). The inferred haplotypes also showed a skewed distribution: DNTM vs. controls, $p=0.01$; PNTM vs. controls, $p=0.0006$; and DNTM vs. PNTM, $p=0.0013$.

Interpretation: The IFNGR1 MPR is highly polymorphic and some of these polymorphisms are significantly associated with nontuberculous mycobacterial infections. This is the first study in which common genetic susceptibility factors have been associated with both disseminated and pulmonary NTM infections.

A novel microsatellite polymorphism in the promoter of human toll-like receptor 2: racial differences, functional implications and possible disease association

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Background: The human toll-like receptor (TLR) family plays crucial roles in both the innate and adaptive immune systems. Among the 10 TLRs identified, TLR2 mediates responses to Gram-positive bacteria, including mycobacteria. We sought functional polymorphisms in the promoter region of TLR2.

Methods: Using direct sequencing of PCR fragments as well as PCR fragment sizing we found a highly polymorphic (GT)n dinucleotide repeat around 120 bp upstream of the TLR2 translational start site. We examined the distribution of each allele in normal donors of African ($n=106$), Caucasian ($n=88$) and Korean ($n=98$) descent. To further understand the functional implications of these polymorphisms, we fused the 1.5 kb of upstream TLR2 containing different lengths of GT repeat to luciferase and examined these in transient transfection under different stimulation conditions.

Results: The numbers of GT repeats varied from 12 to 28, with significant differences in allele distribution between African Americans and Caucasians ($p=0.008$) and between African Americans and Koreans ($p=0.0003$). The promoter activity of the longer alleles [(GT)n ≥20] was significantly more inhibited by 200 IU/ml of interferon-γ ($p=0.002$) than shorter alleles. Interestingly, this inhibition was not observed with 100 IU/ml of GM–CSF. Genotypes consisting of [(GT)n ≥20] repeats were more prevalent in patients with disseminated nontuberculous mycobacterial infection (49%) than in normal controls (24%, $p=0.004$).

Interpretation: Given that TLR2 is critical in the human immune response against invading organisms, functional microsatellite polymorphisms in its promoter may play a role in the pathogenesis of infectious and inflammatory diseases. This particular TLR2 promoter polymorphism is significantly different according to race, appears to be disease associated, and is functionally important and discriminating in terms of in vitro response to interferon-γ, a cytokine critical to the integrity of inflammation.

VIRAL INFECTIONS

A promising synthetic peptide for the design of an Epstein-Barr virus vaccine

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Epstein-Barr virus (EBV) is associated with a deadly complication in organ transplantation, the so-called posttransplant lymphoproliferative disorders (PTLD). Mismatched EBV-seronegative recipients and seropositive donors represent an important risk factor. The management of PTLD is difficult and an interesting preventive strategy would be to immunize the recipients before transplantation. The peptide-based vaccination is advantageous because of the absence of genetic material and its easiness to be manufactured. Nevertheless human leukocyte antigen (HLA) polymorphism in humans is a major obstacle to consider. One of the critical aspects to design such vaccine is to select a synthetic peptide that would be restricted by common HLA alleles and then would be available for more individuals such as EBV-seronegative ones.

Objectives: Our main objectives were to evaluate the capacity of a synthetic peptide corresponding to EBNA2 to induce specific T cells in vitro, to determine which HLA alleles restricted this epitope, and finally to study the reactivity of the specific peptide-induced T cells.

Methods: We obtained peripheral blood mononuclear cells (PBMCs) from ten healthy donors. Then using the peptide TVFYNIPPMPL, we induced all these donors to select the best responders by proliferation assays. Using specific antibodies and HLA-known cells lines, we attempted to determine the HLA alleles for this epitope. Finally, after positively selected CD4+ cells from the best reactive T cell lines, we measured by flowcytometry their capacity to inhibit B cell transformation.

Results: EBV positive serology was confirmed for 6 out of the 10 donors. From these latter, 60% shows specific T cell response to EBNA2 peptide and these were mostly CD4+ T helpers. We found these T cells epitope HLA-DR restricted for 5 donors (HLA-DR1, -DR16, -

DR52 and -DR7) and HLA-DQ (-DQ2) for one donor. Finally we demonstrated that purified peptide-induced CD4+ from one donor could inhibit the growth of EBV-infected autologous B-cells in the early phase of transformation.

Conclusion: This EBNA2 synthetic peptide is very promising to design an EBV vaccine for nonimmune individuals awaiting solid organ transplantation.

The pathogenesis of hepatitis c virus (HCV) is strongly influenced by cytomegalovirus (CMV)

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Background: Virus-virus interactions may influence the pathogenesis of defined human viral infections. Using the clinical setting of liver transplantation (LT), we investigated the potential role that β-herpesviruses may play in the course of HCV infection.

Methods: In 93 consecutive HCV-infected patients followed for a mean of 32 months post-LT, the incidence of CMV, human herpesvirus (HHV) -6 and -7 replication (assessed by PCR on 6 weekly serum samples) and CMV disease were evaluated for their impact on HCV-induced cirrhosis, retransplantation or mortality (primary end point). HCV replication and recurrence of HCV-induced hepatitis and fibrosis (assessed at 16 and 52 weeks post-LT) were evaluated as secondary end points. Statistical analysis was performed using frequency tables, Kaplan Meier estimation, and proportional hazards regression.

Results: The primary end point developed in 28% of patients. Independent of other significant predictors, we observed that CMV (40/91 [35.2%]) but not HHV-6 and -7 reactivation, and CMV disease (23/93 [24.7%]) were associated with the primary end point (Risk Ratio [RR], 1.003; 95% CI, 1.001–1.004; P=0.002; and RR, 4.189; 95% CI, 1.838–9.546; P=0.001, respectively). Early CMV reactivation was strongly associated with mortality (8/32 [25%] vs 0/59 [0%]; P<0.001). CMV disease was also associated with higher fibrosis stage (mean (SD, 1.0±1.19 vs 0.49 (0.83; P=0.04), modified hepatitis activity index (mean±SD, 4.17±3.07 vs 2.96±2.42; P=0.09), and plasma HCV RNA level (mean±SD, 55.71±50.47 vEq/ml vs 36.17±46.95 vEq/ml; P=0.07) at 16 weeks post-LT.

Conclusion: Using the human model of LT, our study demonstrates that the pathogenesis of HCV is strongly and independently influenced by its interaction with CMV but not HHV-6 and -7. The lack of association between HCV and the other β-herpesviruses implies that the impact of CMV on HCV may be mediated by a direct HCV-CMV interaction that is beyond immunomodulation. Importantly, it is observed that even low level CMV replication that does not evolve into clinical disease influences HCV outcome. Thus, aggressive CMV prevention may positively influence the clinical outcome of HCV-infected LT patients.

An X-linked lymphoproliferative disease (XLP) patient with uneventful primary infection and fatal reactivation with Epstein-Barr virus (EBV)

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Background: XLP is an inherited immunodeficiency caused by *SAP* mutations. XLP often manifests itself as fulminant or fatal infectious mononucleosis after primary EBV infection; however, EBV is apparently unrelated to development of lymphoma or dysgammaglobulinemia, other major manifestations of XLP. Thus, how this virus involves the pathogenesis of XLP remains obscure. We experienced a patient with XLP who did not acquire any serious condition after primary EBV infection but eventually developed virus-associated hemophagocytic syndrome (VAHS) and malignant lymphoma (ML) upon reactivation of EBV.

Case Report: A 12-year-old boy presented with a 16-day history of fever and lumbago. Both the patient and his 9 year old brother have been known to have hypogammaglobulinemia of unknown etiology, and were seropositive to EBV with uneventful past medical histories, except for several episodes of otitis media and an episode of pneumonia in the patient. Abdominal CT revealed paraaortic and pelvic lymphadenopathy. Open biopsy of the lymph nodes was performed, which showed necrotic tissue with no malignant cells. He did not respond antibiotics, and subsequently VAHS was diagnosed. Despite intensive therapy including plasma exchange, immunosuppressants and VP16, he deteriorated with development of multiple novel nodular lesions in lungs and bilateral pleural effusions, and died from multiple organ failure. Cytological analysis of pleural fluid was compatible to malignant transformation.

Immunologic Studies: Intracellular staining of SAP protein showed decreased SAP expression in T cells derived from these brothers. Direct sequencing revealed missense mutation S34G in their SAP genes. Plasma EBV load in the patient, determined by real-time PCR, was undetectable on admission, but markedly increased to 2.2×10^5 copies/ml when he developed VAHS. In striking contrast, EBV-specific CTL was barely detectable by MHC-peptide tetramer method.

Conclusions: Despite impaired EBV-specific immunity, primary EBV infection in the patient, and also in his brother, appeared insignificant. Nevertheless, he ultimately developed such serious EBV-associated diseases as VAHS and ML. Thus, role of EBV infection in the pathogenesis of XLP may be complex.